



Newsletter

2009 SGMS Meeting

and

General Assembly

October 29-30, 11:15

Dorint Resort Blüemlisalp

Beatenberg

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Travel information

by car

Basel: (A3/E60)→Zürich→A2/Bern→A6/Interlaken
 Zürich: (A1)→Basel/Bern→A1/Bern→A6/Interlaken
 Genf: (A9/A12)→Lausanne→A1/Bern→A6/Interlaken
 ↑ Beatenberg



by train

train leaves (as of May 13, 2008)

Geneva:

IC 717 at 07:45, track 4
Lausanne at 08:20
Fribourg at 09:04
Bern at 09:26, track 7
 then see *Bern* below

Basel:

IC 1063 at 08:30, track 11 arrives in **Bern** at 09:27, track 3: stay in the train until Thun

Zürich:

IC712 at 08:32
Bern at 09:29, track 4

Bern:

IC 1063 at 09:35, track 3
 arriving in **Thun** at 09:52

Beatenbucht:

Bus 21050 at 10:02
 the bus will arrive in **Beatenbucht** at 10:33
 cable car to **Beatenberg** at 10:44
 it's a 5 minutes walk to the Hotel Dorint from there!

Of course there are trains via Interlaken to Beatenberg.

More info can be found at www.sbb.ch !

SGMS submits for the IMSC 2015 – A joint bid with SFSM and DSM

Following an unsuccessful bid in 2006, the Swiss Group for Mass Spectrometry has decided to resubmit, this time as a joint bid with the French Mass Spectrometry society (SFSM) and the Division of Mass Spectrometry of the Italian Chemical Society (DSM). We again propose Geneva as host city of the 20th IMSC in 2015 with the *Centre International de Conférences Genève* as Conference Site.

We are assisted in the submission process by Corporate Communications of ETH Zurich, a professional event organizer.

The submission is backed by the Swiss Chemical Society. Academia, industry and three of our neighboring countries are represented on the scientific committee.

The members of the scientific committee are:

Renato ZENOBI	ETH Zürich	CHAIR
Marc SUTER	Eawag, Dübendorf	CO-CHAIR President SGMS
Rudolf AEBERSOLD	ETH Zürich	
Günter ALLMAIER	TU Wien, Austria	
Silvia CATINELLA	Chiesi Farmaceutici, Parma, Italy	
Leopoldo CERAULO	University of Palermo, Italy	Vice-President DSM
Julia CHAMOT-ROOKE	Ecole Polytechnique, Paris, France	President SFSM
Laurent FAY	Nestlé Research Center Lausanne	
Eric FOREST	Institut de Biologie Structurale, Grenoble, France	Secretary SFSM
Gianluca GIORGI	University of Siena, Italy	President DSM
Detlef GÜNTHER	ETH Zürich	
Gérard HOPFGARTNER	University of Geneva	
Olivier LAPREVOTE	CNRS, France	IMSF, rep SFSM
Markus STÖCKLI	Novartis Pharma AG, Basel	

Program

Thursday 2009-10-29

Session 1

Chair: Marc Suter, Eawag

11:15 - 11:30

Welcome

11:30 - 12:15

Jukka Jokela, Eawag

Why there are males

12:30 - 14:00

Lunch

Session 2

Chair: Yury Tsybin, EPFL, Lausanne

14:00 - 14:45

Mike Bowers, UCSB

***Amyloid formation:
peptide folding, aggregation and chirality***

14:45 - 15:05

Stefanie Mädler, ETHZ

***Detailed investigations of chemical cross-linking: effects of
binding strengths***

15:05 - 15:25

Markus Macht, Bruker

***Top-down protein analysis using ETD: comparison of results
from a high-resolution QTOF and a novel ion trap***

15:25 - 15:45

Reinaldo Almeida, Advion

***Combining top down and bottom up analyses in a single LCMS
experiment***

15:45 - 16:15

Coffee Break

Session 3

Stephan Brombacher, Novartis Pharma, Basel

16:15 - 16:35

Carsten Krantz, Thermo Fisher

Biomarker workflow from discovery to quantitation

16:35 - 16:55

Michael Affolter, Nestec

Qualitative and quantitative profiling of bovine milk fat globule membrane protein fractions

16:55 - 17:15

Holger Nestler, Eawag

Proteomics analysis of herbicide exposure effects in *Chlamydomonas reinhardtii****Just a breath of a break***

17:20

General Assembly

19:00

Apéro

20:00

Blüemlisalp Dinner Buffet

22:30

Muh-Bar open

Friday 2009-10-30

Session 4

Chair: Laurent Bigler, Uni Zürich

08:30 - 09:15

Marcos Eberlin, UNICAMP

Mass spectrometry EASIER than ever! - Applications of easy ambient sonic-spray ionization mass spectrometry

09:15 - 09:35

Liang Zhu, ETHZ

Towards nanoscale molecular analysis and chemical imaging at atmospheric pressure by near-field laser ablation mass spectrometry

09:35 - 09:55

Susanne Kern, Eawag

Identification of transformation products of organic contaminants in natural waters by computer aided prediction and high-resolution mass spectrometry

09:55 - 10:15

Olivier Scheidegger, Uni ZH

Benefits of the back-flush system for PTV injectors demonstrated by the determination of ethyl glucuronide in hair by gas chromatography negative chemical ionization tandem mass spectrometry

10:15 - 10:45

Coffee Break

Session 5

Chair: Jean-Luc Wolfender, Uni GE, Lausanne

10:45 - 11:30

Reto Stöcklin, Atheris

Venomics: targeted drug discovery and lead optimization using animal venoms

11:30 - 11:50

John Shockor, Waters

Development of a lipidomic platform based on a hybrid quadrupole time-of-flight (QToF) ion-mobility mass spectrometer for both targeted and non-targeted analysis

11:50 - 12:10

Carine Steiner, USZ

HPLC-MS for the investigation of human bile acids

12:10 - 12:30

Anton Kaufmann, Kt Labor ZH

Comparison of selectivity for tandem-MS and high resolution MS in LC: Where is the "crossover point"?

12:30 - 12:35

Closing Remarks

Why there are males



Jukka Jokela

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In several groups of organisms (plants, insects, lizards, fishes) occasional parthenogens (asexual females) are found. Asexual lineages have higher per-capita growth rates because they do not produce males, and do not need to invest in costly mating activities. Therefore it is striking that sexual reproduction dominates the world. Sexual reproduction is a paradox because sexual populations should be vulnerable to invasion by ecologically similar asexual females. One of the hypotheses to explain dominance of sex relates the advantage of sexual reproduction to avoidance of coevolving parasites. As asexual genotypes become common, they may also become disproportionately infected by parasites that have evolved to evade the host's genetically based self-nonsel recognition system. Under this idea, high infection rates in the common asexual clones can periodically favor the genetically diverse sexual individuals (the Red Queen hypothesis), and may promote the short-term coexistence of sexual and asexual populations. Testing the idea requires comparison of sexual and asexual lineages, which are in direct competition. I will review results of a long-term study that examines the clonal dynamics and parasite coevolution in a "mixed" (sexual and asexual) population of freshwater snails (*Potamopyrgus antipodarum*). We found that, within 7-10 years, the most-common clones were almost completely replaced by initially rare clones in two different habitats, while sexuals persisted throughout the study period. The common clones, which were initially more resistant to infection, also became more susceptible to infection to sympatric parasites over the course of the study. Overall, the results support the basic tenets of the Red Queen hypothesis for the maintenance of sex and show that the coevolutionary dynamics predicted by the theory may operate in mixed populations of sexuals and asexuals favoring sexual reproduction.

Amyloid formation: peptide folding, aggregation and chirality



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Amyloid diseases occur because proteins or peptides aggregate and are not effectively dealt with by cellular disposal systems. The mechanism of the folding/aggregation process is undoubtedly dependent on the detailed structure of the peptide or protein but many similarities occur across systems. Here we will use model peptide systems that form amyloid crystals or fibrils but are small enough to model with some degree of rigor. These will include members of the enkephalin family of penta-peptides and the peptide NNQQNY. One of the parameters we will investigate is the chirality of the peptide using the YAGFL enkephalin as an example where all chiral possibilities have been explored. This aspect of the study touches on the known but mysterious fact that all natural peptides and proteins use essentially pure L-amino acids in their primary structures.

Detailed Investigations of Chemical Cross-linking: Effects of Binding Strengths

Stefanie Mädler (1), Markus Seitz (2), John Robinson (2), Renato Zenobi (1)
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Chemical cross-linking in combination with Matrix-Assisted Laser Desorption/Ionization (MALDI) mass spectrometry has emerged as a powerful tool to study non-covalent protein complexes. Nevertheless, there are still many questions to answer: Is there any limitation regarding the binding strengths of a protein complex for which specific chemical cross-linking is possible? Does the amount of detected cross-linked complex correlate with the amount of protein complex in solution? In order to answer these questions we performed systematic cross-linking studies with a non-covalent complex of a coactivator protein (15 kDa) and mutants of an interacting peptide (2.4 kDa) using the cross-linker DiSuccinimidyl Suberate (DSS).

A set of alanine mutants of the chosen peptide (Stat6Y, 794-813) was investigated. The mutants form specific 1 : 1 complexes with the coactivator protein NCoA-1 with a wide range of binding affinities ($KD = 0.03 \dots KD > 25 \mu M$) covering 3 orders of magnitude for KD values. [1] We incubated protein and peptide at equal concentration with DSS and analyzed the reaction mixture with a MALDI-ToF instrument (Reflex III, Bruker Daltonics Inc., Bremen, Germany) retro-fitted with a high-mass detector (HM1, CovalX, Zurich, Switzerland).

A significant amount of cross-linked protein-peptide complex was obtained for all peptide mutants. Thus, the application range of chemical cross-linking can be extended to low affinity complexes. For high affinity complexes, a high amount of cross-linked species was detected, whereas the low-affinity complexes gave a low amount of cross-linked species. In order to prove that the observed cross-linked species is a specific complex, and to investigate the concentration range of specificity, three different peptide mutants were



chosen and their reaction behavior with the chemical cross-linker DSS investigated for three different concentrations. A good correlation between measured and theoretical values was observed. In order to test the specificity of chemical cross-linking against a non-binding peptide, oxidized insulin chain A was selected as a reference compound, since its molecular weight and thus probably its diffusion coefficient is quite similar to the mutated peptides. No significant amount of a cross-linked complex between the coactivator protein and the insulin peptide was observed. Thus, even at higher protein concentrations of 35 μ M, chemical cross-linking requires a specific interaction between the coactivator protein and a peptide.

[1] M Seitz, LT Maillard, D Obrecht, JA Robinson, ChemBioChem 2008, 9, 1318-1322.

Top-down protein analysis using ETD: Comparison of results from a high-resolution QTOF and a novel ion trap

Marcus Macht (1), Arnd Ingendoh (1), Christian Albers (1), Christoph Gebhardt (1), Ralf Hartmer (1), Carsten Stoermer (2), Desmond A Kaplan (2), Oliver Raether, Melvin A Park (2)
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Dedicated MS/MS-techniques for top-down protein analysis are electron-induced fragmentation processes like electron capture (ECD) or electron transfer dissociation (ETD). However, ETD MS/MS spectra of highly charged intact proteins can be rather complicated because of a high number of multiply charged and overlaid fragment ions.

When using ETD in an ion trap, the complexity of the ETD MS/MS-data is reduced by a subsequent proton transfer reaction (PTR) which decreases the high charge states of the fragments. For common ion trap instruments, the charge reduction step is typically optimized to lead to singly and doubly charged fragments. Here, we present a novel, highly sensitive ion trap with an increased resolving power for higher charged ETD-fragments ($z > 4$) which generates an improved sequence coverage for proteins.

The high resolution and accuracy of QTOFs enables to analyze medium size proteins directly with ETD, i.e. without an additional PTR step. With a 50,000 resolving power and < 2 ppm mass accuracy, top-down sequencing can be performed in direct LC coupling, i.e. on the LC timescale. We will discuss advantages of both concepts by data from the novel ion trap and the high resolution QTOF.

Measurements were done on the amazon ion trap and the maxis (Bruker Daltonik). An improved control of the non-linear ejection process and the development of the trap environment support faster scan modes as well as a higher mass resolution. A new ion funnel guide increases the general sensitivity of the ion trap instrument.

The maxis UHR-QTOF was equipped with a nCI source. Reagent and analyte ions were trapped in a hexapole collision cell. After the ETD reaction, product ions are transferred to a



cooling cell where they are stored and extracted to the TOF analyzer. While ions are extracted, the next ETD experiment is ongoing in the collision cell thus maximizing duty cycle. The proteins were either infused by offline nanospray or online separated by LC.

Standard samples Substance-P, Melittin, Beta Casein, and Ubiquitin all showed sequence coverages of 80-100% depending on the number of proline residues, with mass accuracies < 2ppm on all product ions for the maxis. We investigated as well the sequence coverage on recombinant protein QC for samples like interferon and TL 29. Protein standards spiked into plasma were analyzed by online LC-ETD-MS/MS.

Finally, a putative biomarker which was discovered in the Imaging MALDI analysis of human breast cancer tissue was investigated by top-down ETD and could be unambiguously and directly identified.

Combining Top down and Bottom up analyses in a single LCMS experiment

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In this work we use "bottom up" and "top down" analyses by combining online LCMS with nanoESI infusion for the identification of proteins and characterizing their post-translational modifications during the brewing process. The intact mass measurement and primary sequence determination was performed by online UPLC-MS(MS) with simultaneous fraction collection. The "top down" affords the post-translational modification observation after the UPLC-MS run by automated nanoESI infusion of the previously collected fractions. Since nanoESI consumes just a small amount of the analyte, proteolytic digestion was performed on the remaining sample for "bottom up" analyses. This strategy allows us to full characterise proteins involved during brewing, by intact mass determination, PTM characterisation and the corresponding peptide sequence coverage in a single LCMS experiment.

The combination of intact protein chromatography by UPLC with a post column splitting system, showed equal separation power and signal to noise ratio, in comparison with the standard non splitting set up. During the online top down analyses the intact mass could be determined by deconvoluting the charge state envelop. However many proteins have been detected but not identified or with low confidence due to the limited time during the elution. These proteins where then targeted during the offline analyses of the previous collected fractions for fop down MS/MS, but also for intact mass determination of very low abundant species. The offline analyses allowed an unlimited averaging capability and optimisation due to the low sample consumption of nanoESI as well as the specific targeting of post translational modifications. Each protein fraction was then digested and reanalysed by offline bottom up, giving again unlimited time for optimising the condition and averaging during the analyses of the corresponding peptides. The good UPLC separation of the intact proteins



lead that the peptides identified in each fraction can just correspond to the proteins found by the top down approach previously, adding another confidence level to the analyses. Furthermore the complexity in each fraction is reduced in a way that no 2 dimension chromatography run is needed. The combined top down and bottom up results facilitated the interpretation of unknown proteins as well as post translational modifications.

Biomarker workflow from discovery to quantitation

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Proteomics studies were focused on protein identification for many years. The development of new MS technologies and software that supports both identification and quantitation has opened new doors for peptide-based biomarker discovery and validation. Though not standard yet, quantitative proteomics is on its way to routine and the ongoing success in this field will greatly impact our knowledge about onset and progression of diseases. The diagnosis of allograft rejection in kidney transplantation medicine is currently dependent on biopsy. The development of non-invasive biomarkers for transplant rejection would strongly improve the quality of life of the patients and the early detection of rejection. Here we present a workflow based on mass spectrometry on peptides indicative for kidney transplant rejection and disease. We describe a new hybrid of a linear ion trap and a high resolution Orbitrap mass analyzer for protein identification and a state of the art triple quadrupole instrument for quantitation. Endogenous biomarkers in urine are identified by the Orbitrap in the first step of the workflow followed by a validation step performed on a triple quadrupole mass spectrometer making use of the high selectivity and sensitivity of SRM. New features like timed SRM and i-SRM allow up to 3000 transitions to be monitored in one analytical run, enabling the quantitation and validation of very complex mixtures and at the same time confirming the identity of the candidate peptides.



Qualitative and Quantitative Profiling of Bovine Milk Fat Globule Membrane Protein Fractions

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Martin Kussmann

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Milk is a biological fluid of unique quality and complexity. It has co-evolved with mammals and mankind to nourish offspring and contains macro- and micronutrients for growth and development of the newborn. Besides casein and whey, both highly concentrated in milk and extensively exploited by the dairy industry, the milk fat globule membrane (MFGM) represents an important fraction rich in bioactive proteins [1]. In order to better understand and subsequently improve functionality of milk products, e.g. infant formulas, detailed qualitative and quantitative protein knowledge of fractions such as MFGM is required. We present label-free proteome profiling and absolute quantification of selected MFGM proteins comparing two sources. Protein quantification is based on mass spectrometric detection of proteotypic peptides [2] via selected reaction monitoring (SRM) approach recently established in the proteomic field.

Novel aspects: Implementation of SRM-based absolute quantification to characterize bioactive MFGM proteins for nutritional applications.

[1] K Dewettinck, R Rombaut, N Thienpont, TT Le, K Messens, J Van Camp, *Int Dairy J*, 2008, 18, 436-457.

[2] P Mallick, M Schirle, SC Chen, MR Flory, H Lee, D Martin, J Ranish, B Raught, R Schmitt, T Werner, B Kuster, R Aebersold, *Nature Biotech* 2006, 25, 125-131.

Proteomics analysis of herbicide exposure effects in Chlamydomonas reinhardtii

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The application of proteomics in ecotoxicology aims at linking the physiological effects and modes of action of toxicants to variations in the target organism's proteome. For our study we chose the single celled green alga and model organism *Chlamydomonas reinhardtii* and a selection of model herbicides, each representing one specific mode of action. The assessment of their physiological effects on the green alga reveals suitable exposure conditions to prepare samples for the proteomic analysis. Of special interest are conditions around the lowest observed effect concentration. The entire approach consists of proteomic profiling by 2D-LC-MS/MS in combination with the search engines OMSSA and X!Tandem, and the subsequent statistical analysis using G-test.

A comparison of protein identifications between control and paraquat exposure conditions (6h) showed variations of protein compositions in a wide range of proteins, metabolic pathways and protein complexes which clearly requires a more detailed analysis. 0.066 μM paraquat for instance already caused a drop in the abundance of various photosystem components and Calvin cycle enzymes, but lead to an increased abundance of proteins involved in other cellular functions such as protein modification. 0.66 μM paraquat reversed some of the alterations of the lower concentration, kept or strengthened others, and showed clear signs of cellular decay in accordance with the physiological observations. The work presented demonstrates that a qualitative and quantitative assessment of the stress effects on the proteome level as well as the identification of protein markers for the applied exposure conditions is possible.

Mass spectrometry EASIER than ever! -Applications of easy ambient sonic-spray ionization mass spectrometry (EASI-MS)



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We have introduced recently a new desorption/ionization for ambient mass spectrometry: easy ambient sonic-spray ionization (EASI). When EASI is applied, simple and efficient desorption and ionization of analytes occurs at ambient conditions owing to the bombardment of the surface containing the analyte with a supersonic cloud of very tiny charged droplets. An advantage of EASI-MS is the use of neither heating nor (high) voltages at the spray capillary. EASI-MS provides the cleanest mass spectra (as compared to related techniques) with few solvent cluster ions and the softest ionization (no or reduced dissociation) with enough abundant analyte signals even for EASI-MS/MS. These features facilitate therefore the detection of all components and impurities in a 1:1 component – ion fashion. The high-velocity supersonic EASI spray also facilitates deep matrix penetration thus providing quite homogenous sampling and long-lasting ion signals, with no electro or photochemical interferences. EASI provides therefore a very friendly environment in which to perform ambient mass spectrometry. In this talk we will show examples of the applications of EASI-MS such as for the analysis of drug tablets and on-tablet drug stability, for typification and counterfeiting detection of perfumes, vegetable oils and (bio)fuels, counterfeit money bills, ink aging, death dating, and the analysis of environmental body fluids using semi-permeable membranes.

Towards nanoscale molecular analysis and chemical imaging at atmospheric pressure by near-field laser ablation mass spectrometry

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The need for analytical techniques capable of obtaining both chemical and topographic information of samples at the nanoscale becomes urgent when it is recognized that most traditional chemical analysis methods are far from achieving nanoscale resolution and most traditional techniques used for nanoscale characterization give virtually no chemical information.

To achieve chemical analysis with nanoscale resolution at atmospheric pressure, an instrument was developed in our laboratory that couples mass spectrometry (MS) to laser ablation (LA) sampling via near-field (NF) techniques. A UV laser pulse is delivered through an optical near-field probe to ablate the analyte. The near-field ablation plume is then transferred through a capillary into an ion-trap/time-of-flight hybrid mass spectrometer. [1] In this fashion molecular analysis of the sample surface become possible where the spatial resolution is limited by the near-field probe aperture and utilized laser energy.

With this instrument, spatially resolved molecular analysis yielding full mass spectral information for anthracene samples at atmospheric pressure could be demonstrated for the first time with a lateral resolution in the low μm range [2]. However, although nanoscale sample craters can be produced routinely, no full molecular mass spectra of ablated material from craters of $\approx 1 \mu\text{m}$ diameter have been acquired yet by this approach. Some of the pressing questions are thus how much of the ablated material is transported into the mass spectrometer, and in what form. Therefore, material redeposition from laser ablation of molecular solids on the near-field tip's surface, and on a capture plate located before the ion trap chamber was characterized with scanning electron microscopy (SEM), allowing some fundamental insight into the near-field LA event, such as the form of ablated products, and



the propagation height and direction of the NF LA plume [3]. Another important point to be clarified is the suction efficiency through the capillary with help of the pressure difference. This question was explored by simulating NF laser plume suction on the far-field scale and imaging the plume propagation with a suction capillary nearby. The influences of the capillary-crater distance, the gas flow and the laser profile on the plume propagation have been systematically investigated. By modifying our instrumental setup according to the above-mentioned findings, a characterization of the improved instrument performance with different samples will be presented. Ultimately, this setup will allow mass spectral imaging on the nanoscale at atmospheric pressure.

- [1] PD Setz, TA Schmitz, R Zenobi, *Rev Sci Instrum* 2006, 77, 024101.
- [2] TA Schmitz, G Gamez, PD Setz, L Zhu, R Zenobi, *Anal Chem* 2008, 80, 6537.
- [3] L Zhu, G Gamez, TA Schmitz, R Zenobi, *Anal Bioanal Chem* 2009, DOI 10.1007/s00216-009-2919-1.

Identification of transformation products of organic contaminants in natural waters by computer-aided prediction and high-resolution mass spectrometry

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Juliane Hollender (1,2)

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Transformation products (TPs) of organic contaminants in aquatic environments are still rarely considered in water quality and chemical risk assessment, although they have been found in concentrations that are of concern. Since many different TPs can potentially be formed in the environment and analytical standards are typically lacking for these compounds, knowledge on the prevalence of TPs in aquatic environments is fragmentary. In this study, an efficient procedure was therefore developed to comprehensively screen for large numbers of potential TPs in environmental samples. It is based on a target list of plausible TPs that has been assembled using the University of Minnesota Pathway Prediction System (UM-PPS) for the computer-aided prediction of products of microbial metabolism and an extensive search for TPs reported in the scientific literature. The analytical procedure for screening of the compounds on the target list has been developed to allow for the detection of a broad range of compounds in complex environmental samples in the absence of commercially available reference standards. It includes solid phase extraction with broad enrichment efficiency, followed by liquid chromatography and tandem mass spectrometry with high mass resolution and accuracy. The identification of target TPs consisted of extracting the exact mass from the chromatogram, selecting peaks of sufficient intensity, checking the plausibility of the retention time, and interpreting mass spectra. The procedure was used to screen for TPs of 52 pesticides, biocides, and pharmaceuticals in seven representative surface water samples from different regions in Switzerland. Altogether 19 TPs were identified, including both some well-known and commonly detected TPs, and



some rarely reported ones (e.g., biotransformation products of the pharmaceuticals venlafaxine and verapamil, or of the pesticide azoxystrobin). Overall, the rather low number of TPs detected suggests that TPs may not pose a problem of unexpected magnitude for aquatic resources.

Benefits of the back-flush system for PTV injectors demonstrated by the determination of ethyl glucuronide in hair by gas chromatography negative chemical ionization tandem mass spectrometry

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Ethyl glucuronide (EtG) is a direct metabolite of ethanol (phase II metabolite). As EtG accumulates in hair it is used as a retrospective long-term marker for chronic alcohol consumption and to demonstrate teetotalism, respectively. Interest in the determination of EtG in human hair is currently growing in clinical and forensic medicine.

Gas chromatography connected to negative chemical ionization tandem mass spectrometry (GC-NCI-MS-MS) is the method of choice for EtG in hairs, as it combines the advantages of separation power and high selectivity. Tandem mass spectrometry allows the monitoring of several transitions for identification and quantification of the EtG present at pg/mg level in hair.

The sample preparation consists of the extraction of EtG from a homogenized strand of hair followed by a solid phase extraction (SPE) cleanup. EtG and the internal standard d5-EtG are derivatized with pentafluoropropionic acid anhydride (PFPA) prior to injection. However, the prepared samples still contain a significant amount of heavy matrix often leading to rapid loss in intensity and sensitivity. Consequently, maintenance of the GC-MS system is necessary after analysis of only a small series of samples.

The back-flush system is an option available for programmable temperature vaporizing (PTV) injectors in gas chromatography. It consists of a T-connector between a precolumn and the analytical column. Flow regulation allows the transfer of a selected part of the sample into the analytical column as well as the back-flush of unwanted fractions pass the injector.



EtG in hair was determined by GC-NCI-MS-MS with and without the back-flush system. With the back-flush system on, the later eluting part of the sample was restrained from entering the analytical column, the carrier gas flow was switched to back-flush and the later fraction was eliminated from the precolumn and blown out of the injection system, thus cleaning both devices and preventing contamination.

Compared to standard operation without PTV and back-flush, applying the back-flush system results in a better robustness as well as shortening of the GC runtime since column bake out is not needed anymore. The required GC maintenance (exchange of liner and precolumn) was reduced by factor of five. The lifetime of the analytical column was prolonged by a factor of two to three and source cleaning was reduced by a factor of three. The results clearly demonstrate the advantages of the back-flush system saving time and costs. Ongoing optimization experiments with other tools of the PTV injector such as the PTV solvent split show promise.

Venomics: targeted drug discovery and lead optimization using animal venoms



Reto Stöcklin

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Venoms are rich mixtures of peptides and proteins evolved by Nature to catch and digest preys or for protection against predators. They represent extensive sources of potent and selective bioactive compounds to discover and develop new drugs. Conventional bioactivity-guided strategies are time consuming and require large amounts of material. In contrast, state-of-the-art proteomic, transcriptomic and post-genomic technologies coupled to bioinformatics can swiftly generate abundant and valuable data using minimal sample amounts.

In a typical drug discovery project, we use our databases to select venoms offering higher chances to generate hits for a given target. The venoms are pre-fractionated using specific methods and natural libraries are made ready for bioassays (HTS). The fraction composition is often investigated in parallel on our mass spectrometry and bioinformatics platform with database matching. Stringent criteria are used to pick out fractions for deconvolution, and hits are synthesized at an early stage to generate synthetic libraries of bioactive candidates. After deeper evaluations, selected leads undergo optimization through drug design and structure-function studies. Here again, we developed an original approach: our platform is designed to screen related venoms and other organisms to identify natural analogues of the lead compound in order to exploit what nature has optimized through million years of natural selection.

Our bioactivity-guided, structure-driven and biocomputing-assisted Venomics strategies will be illustrated through the discovery of novel sarafotoxins (endothelin-type peptides), original protease inhibitors (pHpG's), bradykinin-potentiating peptides (BPP's) and novel antimicrobial peptides. The presentation will also focus on "CONCO", the first fully integrated Venomics project, which is devoted to cone snails. The genome, transcriptome and proteome of *Conus consors* are currently exhaustively studied. The biological activities of natural and synthetic libraries are investigated. Selected peptides are further characterized in vivo and their potential as novel biopharmaceutical drug candidates is evaluated. Additionally, the biodiversity, ecology and molecular evolution of a wide range of venomous gastropod species are studied. We believe that our unique techniques, combining mass spectrometry to in silico data and text mining strategies, are a straightforward discovery approach for novel biomolecules from animal venoms and other natural sources.

CONCO, the cone snail genome project for health, is funded by the European Commission: LIFESCIHEALTH-6 Integrated Project LSHB-CT-2007-037592. We are grateful to the governments of New Caledonia and French Polynesia.

References: See http://www.atheris.com/sel_lit.php.

Development of a lipidomic platform based on a hybrid quadrupole time-of-flight (QToF) ion-mobility mass spectrometer for both targeted and non-targeted analysis

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Lipids are broadly defined as any fat-soluble (lipophilic), naturally-occurring molecule, and include fats, oils, waxes, cholesterol, sterols, monoglycerides, diglycerides triglycerides, and phospholipids. The main biological functions of lipids are energy storage, as structural components of cell membranes, and as important signaling molecules. Mass spectrometry plays an important role in the study of lipid biochemistry. For example, the information it provides can be critical to understanding the mechanism of pathogenesis for diseases which are linked to abnormal physiological levels of certain lipids including atherosclerosis and diabetes.

Recent technological advances have yielded hybrid instruments such as the quadrupole time-of-flight (QToF) ion-mobility mass spectrometer which is an ideal platform for lipid analysis. This instrument possesses clear analytical advantages over conventional tandem quadrupole and linear ion-traps in full scan sensitivity, mass accuracy, spectral resolution and fragmentation. It can also provide an added dimension of separation to the analysis via ion-mobility. With the hybrid QToF it is possible to conduct class specific precursor and neutral loss acquisitions over a single experimental run using an instrument acquisition mode called elevated-energy mass spectrometry (MSE). MSE is a term which is used to describe a strategy which performs data-independent fragmentation experiments. The acquisition mode is divided into two parallel, alternating scan functions. In both functions the Q1 mass filter is operated in a wide band rf mode to pass all ions; however, in the first function the collision cell is operated at low collision energy so that essentially only parent ion mass information is recorded, while in the second function the collision cell is ramped over an elevated energy



range to produce product ion information. Related parent and product ion information is chromatographically time-aligned in a post acquisition processing step. Both parent and product ions are measured in accurate mass mode. The MSE data acquisition rate is adjusted to collect a minimum of seven data points in both low and elevated energy mode across each chromatographic to provide an accurate quantitative assessment of each component. This Acquisition strategy provides a 100% duty-cycle accurate mass analysis of all detectable parent and product ion information in a complex mixture. The exact mass information obtained provides a more definitive descriptor of the molecule and is very important to removal false positives.

The specificity and reliability of this strategy allows us to use this technology as a 'shotgun' LC/MS approach to search for phospholipids, diacylglycerides (DAG) and triacylglycerides (TAG) in an unbiased robust manner. Applying this technology allowed the specific detection of intact molecular ions, precursor ions and neutral losses in either positive or negative ionization mode that upon collision-induced dissociation generated characteristic diagnostic fragment and neutral loss ions. For instance, in positive ion mode phosphocholines and sphingomyelins are readily detected as protonated molecular cations. Upon CID they both generate the m/z 184.0733 fragment ion corresponding to the polar head group, $[(CH_3)_3N+C_2H_4OP(OH)_2]^+$. However, ions yielding structural information about the fatty acid side-chains are of low abundance and typically other solvents such as LiOH (post column or addition to the mobile phase) are used to obtain structural information about them. Utilizing the unique characteristics of the ion-mobility sector of the instrument we are able to perform time-aligned parallel fragmentation experiments which yield fragment ions that facilitate assignment of the fatty-acid side chains. In this paper, a robust LC/MS platform for detection and characterization of multiple classes of phospholipids, diacylglycerides and triacylglycerides is described and illustrated with data from extracted human plasma samples.

HPLC-MS for the investigation of human bile acids

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Introduction: Bile acids are the major degradation products of cholesterol and they undergo considerable structural modification through hepatic and intestinal metabolism. They are biologically important as mediators of dietary lipid absorption as well as ligands of the nuclear receptor farnesoid X receptor (FXR) by which they regulate lipid and carbohydrate metabolism. Since bile acids are reabsorbed through an efficient enterohepatic circulation, additional assessment of their precursor 7 α -hydroxy-4-cholesten-3-one (C4) is used to provide information about the direct input of de novo biosynthesis out of cholesterol in contrast to the input coming from reabsorption of bile acids from the intestine. Our interest lies in determining the diagnostic and/or prognostic significance of bile acid quantification in patients suffering from various diseases including metabolic syndrome. However, it is well known that bile acid levels in serum undergo a diurnal variation, which may lead to different concentration findings in the same subject depending on the blood sampling time.

Methods: The 15 major human bile acids as well as their precursor C4 were quantified using two methods based on LC-MS since this technique allows sensitive quantification of compounds present in the micromolar range in serum. Moreover, the bile acid pool contains several isomers, which vary in their ability to activate the FXR. Differentiated quantification of these compounds is achieved by combining chromatographic separation with the selectivity of mass spectrometry.

Results: The circadian rhythm of the aforementioned compounds was described in four healthy subjects during 24 hours in 1 hour intervals. Preliminary data show considerable interindividual variation in concentrations of the unconjugated bile acids, whereas the concentrations of glycine- as well as taurine-conjugates seem to depend on food intake, the latter observation being in agreement with previously reported data.

Conclusion: While previous studies mostly rely on quantification of total or unconjugated bile acids, our methods allow the description of the differentiated bile acid circadian rhythm. These results should allow us to define the time interval with the least intraindividual fluctuations and, therefore the best point in time to take blood samples in order to obtain stable results.



Comparison of Selectivity for Tandem-MS and High Resolution MS in LC: Where is the "crossover point"?

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Targeted drug analysis in biological matrices like plasma or tissue extracts, as well as drug discovery and the study of drug metabolism has been the domain of LC-MS-MS. This analytical technique provides a very high level of selectivity and sensitivity. Consequently LC-MS-MS has also become the standard tool for residue analysis in food (e.g. veterinary drugs or pesticides).

An alternative way to obtain high selectivity is the use of high resolution (HR) instruments, like Time of Flight or Orbitrap MS. Such instruments produce full scan data and can potentially detect any ionisable analyte. This provides a significant advantage over MS-MS instrumentation, which monitors only the pre-programmed transitions. However, the lack of selectivity has prevented a wide acceptance of HR-MS in routine laboratories.

This issue has been systematically investigated by comparing an LC-MS-MS versus a nonhybrid LC-Orbitrap. The likelihood that a complex matrix produces potentially interfering signals which are caused by co-extractives, was thoroughly studied.

Different blank matrices extracts (muscle, kidney and honey) were injected, chromatographed and a large number of dummy MS-MS transitions were monitored. These dummy transitions were the product of a random number generator. Mass range restrictions were defined, so that "meaningful" dummy values for precursor and product ions resulted. All observed chromatographic peaks were integrated. On the other hand, a large number of dummy exact mass traces with mass windows widths of only a few mDa were extracted from Orbitrap full scan datafiles. Again, different matrices were tested, while the chosen MS resolution ranged from 10'000 to 100'000 Full Width at Halve Maximum (FWHM). The observed peak areas were compared to those obtained from the MS-MS experiments. A

direct comparison between the two data sets was made possible by transposing these areas in "analyte equivalents". Therefore, an average MS-MS and Orbitrap sensitivity was determined by averaging the response for 10 different compounds (antibiotics) present at equal concentrations in a pure standard solution. The peak areas related to the dummy transitions and dummy exact mass traces were divided by these corresponding average responses. The resulting "analyte equivalents" related to the tested two MS technologies were compared.

The paper will discuss the MS resolution needed for different matrices and analytical problems. It will also try to elucidate the selectivity crossover-point for the two techniques.



2009 General Assembly of the SGMS

Thursday October 29 , 2009

17:30 h

Dorint Resort Blüemlisalp, Beatenberg

Agenda

1. Nomination of the scrutineers.
2. Approval of the minutes of the 2008 general assembly.
3. President's report and its approval.
4. Treasurer's report.
5. Auditor's report and approval of treasurer's and auditor's report.
6. Decision on the 2010 membership fee.
7. Admission of new members.
8. Election of the auditors
9. SGMS homepage - M Suter.
10. Update on the 2015 IMSC bid.
11. Individual proposals.
12. Miscellaneous.

Individual proposals must be sent to marc.suter@eawag.ch before October 15, 2009.

The President, Marc Suter

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